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# Interstitial Deletion of Chromosome 1q [del(1)(q24q25.3)] Identified by Fluorescence In Situ Hybridization and Gene Dosage Analysis of Apolipoprotein A-II, Coagulation Factor V, and Antithrombin III

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**We report on a 12-month-old Japanese boy with an interstitial deletion of the long-arm of chromosome 1 and meningomyelocele, hydrocephalus, anal atresia, atrial septal defect, left renal agenesis, bilateral cryptorchidism, talipes equinovarus, low birth weight, growth/developmental retardation, and many minor anomalies. By conventional GTG-banding, his karyotype was first interpreted as 46,XY,del(1)(q23q24), but it was corrected as 46,XY.ish del(1)(q24q25.3) by fluorescence in situ hybridization using 11 known cosmid clones as probes. His serum levels of apolipoprotein A-II (gene symbol: APOA2, previously assigned to 1q21-q23) and coagulation factor V (F5, 1q21-q25) were normal, while serum concentration and activity of antithrombin III (AT3, 1q23-q25.1) was low. The results indicated that localization of APOA2 and F5 are proximal to the deleted region and AT3 is located within the deletion extent in the patient. Am. J. Med. Genet. 68:207–210, 1997** 

**KEY WORDS: interstitial deletion of chromosome 1; fluorescence in situ hybridization; apolipoprotein A-II; coagulation factor V; antithrombin III; gene mapping**

## INTRODUCTION

Reports of interstitial deletion of the long-arm of chromosome 1 are scarce. Only 11 cases of monosomy for 1q21-q25 have been published [reviewed by Franco et al., 1991]. The clinical manifestations reported included low birth-weight, growth and developmental delay, hypotonia, microbrachycephaly, cleft palate, apparently low-set and posteriorly angulated ears, small hands and feet, fifth finger clinodactyly, bilateral transverse palmer crease, external genital abnormalities, and occasional cardiac and kidney abnormalities. Although most of their deletions were identified cytogenetically, a deletion extent in one case was confirmed with Southern blot analysis.

This report deals with a patient with an interstitial 1q deletion whose breakpoints were identified precisely by fluorescence in situ hybridization (FISH). Some of the clinical manifestations of the patient are distinct from those reported previously.

## MATERIALS AND METHODS

### Clinical Report

The subject was the second son of healthy parents of Japanese ancestry. The mother had an induced abortion during the previous marriage. The father was 36 years old and the mother 28 years old at the time of the patient's birth. Maternal serum α-fetoprotein level was 272.3 ng/ml (normal mean in the Japanese, 275.3 ng/ml; normal range, 97.5–473.8 ng/ml) at 33 weeks of pregnancy. At the same time, cerebral ventricular dilatation, spina bifida, and lower limb dysgenesis in the fetus were diagnosed by ultrasound scanning. Cesarean section was performed at 39 weeks-6 days' gestation because of the fetal anomalies. The birth weight was 2,342 g (<3rd centile), crown-heel length was 41 cm (<3rd centile), head circumference (OFC) was 35 cm (>75th centile), and chest circumference 37 cm (>97th centile according to the statistical data of the 1990 National Growth Survey on Infancy and Childhood by the

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Ministry of Health and Welfare of Japan). At birth, there were multiple major anomalies, including meningomyelocele, hydrocephalus, anal atresia, atrial septal defect, left renal agenesis, bilateral cryptorchidism, and talipes equinovarus. There were also multiple minor anomalies, including frontal bossing, apparently low-set and malformed ears (angular overlapping helix and prominent antitragus), high-arched palate, bifid uvula, fifth-finger clinodactyly, bilateral transverse palmer crease, and deformity of the penis (Fig. 1). Colostomy and ventriculoperitoneal shunt operation were performed. The parents were given genetic counseling and are taking care of the patient at home. The patient is now 17 months old and has marked developmental delay. GTG-banding chromosome analysis demonstrated a 46,XY,del(1)(q23q24) (Fig. 2). Karyotypes of the parents were normal.

### FISH Analysis

FISH was performed on the patient's metaphase chromosomes by using 11 known cosmid clones supplied by the Japanese Cancer Resources Bank (JCRB)-Gene (Tokyo, Japan) as probes in order to identify precise breakpoints of the deletion (Fig. 3). These cosmids included cYS1-15, cYS1-40, cYS1-41, cYS1-54, cYS1-68, cYS1-94, cYS1-162, cYS1-178, cYS1-230, cYS1-291, and cYS1-411, all having been mapped to various regions of 1q [Kugoh et al., 1995] (Fig. 3). Methods for chromosome preparation, probe labeling, hybridization, and washing were according to Takahashi et al. [1990, 1991]. In order to eliminate FISH signals from interspersed repetitive DNA sequences in the cosmid probes, 50 times the excess amount of unlabeled human Cot-1 DNA (GIBCO BRL, NY) was added. A laser scanning microscope with epifluorescence optics (LSM-GB200, Olympus, Tokyo) was used for the detection of FITC signals on propidium iodide-stained chromosomes.

Fig. 1. Patient at birth.

Fig. 2. GTG-banded chromosomes 1 of the patient. Arrow indicates deleted chromosome.

### Assays for Apolipoprotein A-II, Coagulation Factor V, and Antithrombin III

Since gene loci for apolipoprotein A-II, coagulation factor V, and antithrombin III have been assigned to 1q21-q25 [Bruns and Dracopoli, 1995], gene dosage analysis was performed in the patient. Serum levels of apolipoprotein A-II and coagulation factor V were measured with the turbidimetric immunoassay [Rifai and Silverman, 1986] and with the chromogenic PT method [Dati et al., 1987], respectively. Serum concentration of antithrombin III was measured immunologically [Nilsson, 1975] and its activity with the chromogenic substrate method [Scully and Kakkar, 1977]. The values in the parents were not available.

## RESULTS

### Identification of Breakpoints by FISH

FISH with seven cosmid clones (cYS1-94, cYS1-40, cYS1-68, cYS1-54, cYS1-15, cYS1-41, and cYS-291) gave FITC signals on both homologous chromosomes 1, indicating that these loci are outside the deletion extent (Fig. 4a). On the other hand, when using cYS1-411, cYS1-178, cYS1-162, and cYS1-230 as probes, FITC signals appeared on only one of the homologues 1 (Fig. 4b). Since these four loci had been assigned to 1q24-q25.3 [Kugoh et al., 1995], the breakpoints that had first been identified by 550-stage GTG-banding were corrected to be 1q24 and 1q25.3 (Fig. 3). Thus, the karyotype of the patient was finally interpreted as 46,XY.ish del(1)(q24q25.3)(cYS1-94+,cYS1-40+,cYS1-68+,cYS1-411-,cYS1-178-,cYS1-162-,cYS1-230-,cYS1-54+,cYS1-15+,cYS1-41+,cYS1-291+) [Mitelman, 1995].

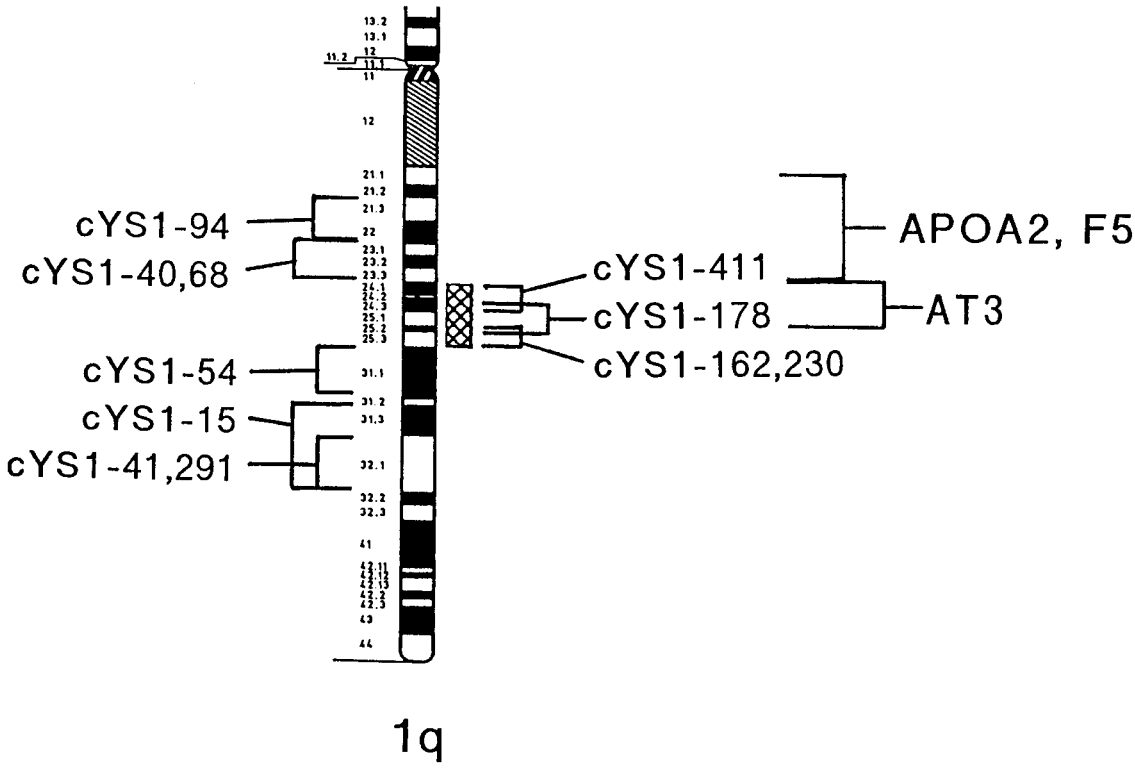


Fig. 3. Localization of the cYS1 series, APOA2, F5 and AT3, and deleted region of the patient (cross-hatched box). Clones on the left and on the right indicate those with which FISH signals are detected on both homologous chromosomes 1, and on only one homologue 1, respectively.

### Gene Dosage Analysis

Serum level of apolipoprotein A-II at 1 month of the patient age was 20 mg/dl, being within normal range (normal range among 100 adults, 20–40 mg/dl; reference values for three neonates/infants in our laboratory, 24–40 mg/dl). Activities of coagulation factor V measured at 3 months and at 12 months were 115% and 118%, respectively. Since the values for normal infants are comparable to those for adults (normal range among 63 adults, 50–150%), the activity in our patient was within normal limits. Antithrombin III concentration measured at age 12 months was low (9.1 mg/dl) and its activity at 3 months and at 12 months was also low (42% and 40%, respectively). The concentration among 100 normal adults ranges between 23 and 34 mg/dl and the activity ranges between 79 and 121%. The values in infants aged 12 months or less are 90% of those for adults, and they reach the adult level after 1 year [Nilsson 1975]. Thus, the antithrombin III level in our patient was about a half of the normal value.

## DISCUSSION

The FISH technique is a powerful tool for gene mapping and widely used in many applications. We use FISH as a clinical diagnostic tool to determine the precise deletion breakpoint. As a result, the breakpoints diagnosed by conventional cytogenetic methods were wrong and corrected to be 1q24 and 1q25.3 by FISH.

Patients with 1q-deletion are classified into three groups: monosomies for 1q21-q25, 1q25-q32, and for 1q42-qter [Schinzel, 1984]. Since the deletion of our patient involved a 1q24-q25.3 region, he belongs to the first group of patients. Among the clinical manifestations in the 11 previously reported cases of the first group deletion, congenital heart disease, kidney and genital abnormalities, and talipes equinovarus were common to them [Franco et al., 1991]. Our patient represented all these common manifestations, and had three additional anomalies: meningomyelocele, hydrocephalus, and anal atresia. In addition, he was clinically distinct from patients with 1q25-q32 deletion-associated prune-belly and Potter sequences [Scarbrough et al., 1988]. Since the deletion of our patient encompassed a 1q24-q25 region and APOA2, AT3, and F5 were said to be assigned to a proximal half region of 1q, dosage analyses of the three genes were performed. As a result, the previous assignment of APOA2 to 1q21-23 [Bruns and Dracopoli, 1995; Dracopoli et al., 1991; Knott et al., 1984] was confirmed, because our patient had a normal serum level of apolipoprotein A-II (Fig. 3). On the other hand, the finding of a normal serum level of coagulation factor V in the patient demonstrated that F5 is located outside his deletion extent. F5 was previously assigned to 1q21-q25 by isotopic in situ hybridization [Wang et al., 1988]. Thus, our findings could narrow the locus into 1q21-q23. Likewise, AT3 was

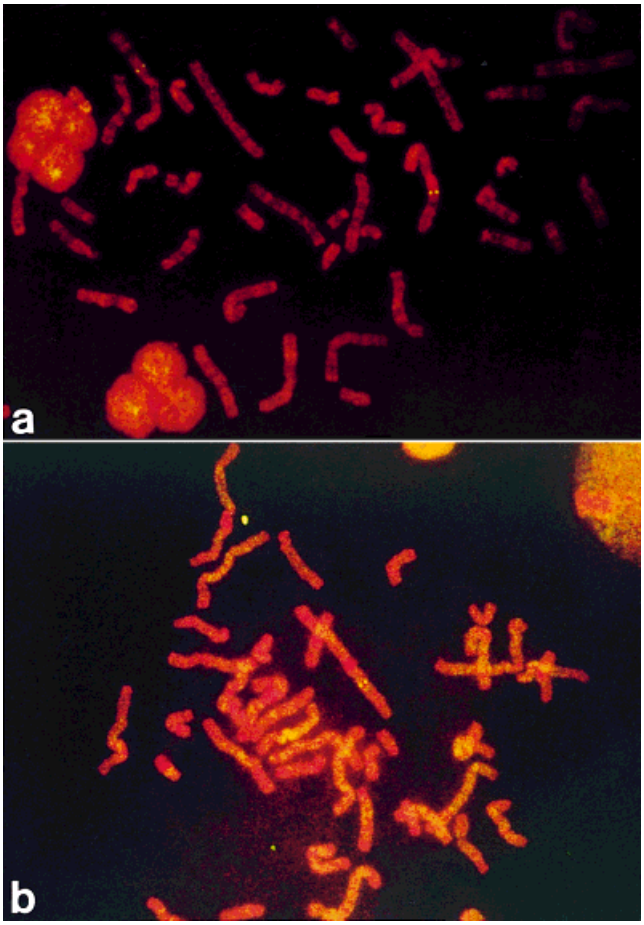


Fig. 4. FISH on the patient's chromosomes. The cYS1-94 signals are seen on both homologous chromosomes 1 (**a**), while cYS1-114 signals are on only one of homologues 1 (**b**).

previously assigned to 1q23-q25.1 [Bock et al., 1985], but the low activity and low serum concentration of antithrombin III could assign AT3 within the deletion, 1q24-q25.1 (Fig. 3), although enzymatic levels may have been affected either by a feedback mechanism or by an interaction with other genes. Also, it cannot be ruled out that the patient actually had two copies of AT3, representing a low activity.

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## REFERENCES

Bock SC, Harris JF, Balazs I, Trent JM (1985): Assignment of the human antithrombin III structural gene to chromosome 1q23-25. Cytogenet Cell Genet 39:67–69.

Bruns GA, Dracopoli NC (1995): Report of the committee on the genetic constitution of chromosome 1. In Cuticchia AJ (ed): "Human Gene Mapping 1994: A Compendium." Baltimore: Johns Hopkins, pp 148–185.

Dati F, Barthels M, Conard J, Flückinger J, Girolami A, Hänseler E, Huber J, Keller F, Kolde HJ, Müller-Berghaus G, Samama M, Thiel W (1987): Multicenter evaluation of a chromogenic substrate method for photometric determination of prothrombin time. Thromb Haemost 58:856–865.

Dracopoli NC, O'Connell P, Elsner TI, Lalouel J-M, White RL, Buetow KH, Nishimura DY, Murray JC, Helmus C, Mishra SK, Donis-Keller H, Hall JM, Lee MK, King M-C, Attwood J, Morton NE, Robson EB, Mahtani M, Willard HF, Royle NJ, Patel I, Jeffreys AJ, Verga V, Jenkins T, Weber JL, Mitchel AL, Bale AE (1991): The CEPH consortium linkage map of human chromosome 1. Genomics 9:686–700.

Franco B, Lai L-W, Patterson D, Ledbetter DH, Trask BJ, van den Engh G, Iannaccone S, Frances S, Patel PI, Lupski JR (1991): Molecular characterization of a patient with del(1)(q23-q25). Hum Genet 87:269–277.

Knott TJ, Priestley LK, Urdea M, Scott J (1984): Isolation and characterization of a cDNA encoding the precursor for human apolipoprotein AII. Biochem Biophys Res Commun 120:734–740.

Kugoh H, Nakagawa Y, Mitsuya K, Mita T, Suzuki M, Suzuki N, Uejima H, Yuasa Y, Oshimura M (1995): Isolation and mapping of 186 new DNA markers on human chromosome 1. Genomics 27: 207–210.

Mitelman F (ed): "ISCN 1995. An International System for Human Cytogenetic Nomenclature." Basel: Karger, pp 94–99.

Nilsson ACT (1975): Antithrombin in infancy and childhood. Acta Padiatr Scand 64:624–628.

Rifai N, Silverman LM (1986): Immunoturbidimetric techniques for quantifying apolipoproteins CII and CIII. Clin Chem 32: 1969–1972.

Scarbrough PR, Files B, Carroll AJ, Quilan RW, Finley SC, Finley WH (1988). Interstitial deletion of chromosome 1 [del(1)(q25q32)] in an infant with prune belly sequence. Prenat Diagn 8:169–174.

Schinzel A (1984): "Catalogue of Unbalanced Chromosome Aberrations in Man." New York: Walter de Gruter, pp 71–75.

Scully MF, Kakkar VV (1977). Methods for semi micro or automated determination of thrombin, antithrombin, and heparin cofactor using the substrate, H-D-Phe-Pip-Arg-p-nitroanilide-2HCl. Clin Chim Acta 79:595–602.

Takahashi E, Hori T, O'Connell P, Leppert M, White R (1990): R-banding and nonisotopic in situ hybridization: Precise localization of the human type II collagen gene (COL2A1). Hum Genet 86:14–16.

Takahashi E, Yamauchi M, Tsuji H, Hitomi A, Meuth M, Hori T (1991): Chromosome mapping of the human cytidine-5'-triphosphate synthetase (CTPS) gene to band 1p34.1-p34.3 by fluorescence in situ hybridization. Hum Genet 88:119–121.

Wang H, Riddell DC, Guinto ER, MacGillivray RTA, Hamerton JL (1988): Localization of the gene encoding human factor V to chromosome 1q21-25. Genomics 2:324–328.